Measurements of absolute concentrations of NADH in cells using the phasor FLIM method.

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Public Summary:
This paper describes studies developing a method to measure the absolute concentration of a key metabolic enzyme in living cells. The method allows scientists to not determine the absolute concentration of that enzyme in living cells and, therefore, to determine the health of cells in living tissues and to distinguish different cells types (with different metabolic profiles) from each other in living tissues. The technology is a further development of methods to study stem cells in living tissues in order to study their biology, to determine the health of living tissues, to distinguish normal cells from abnormal (perhaps cancerous) cells and to help define the risks to normal cells in adults and embryos of environmental chemicals.

Scientific Abstract:
We propose a graphical method using the phasor representation of the fluorescence decay to derive the absolute concentration of NADH in cells. The method requires the measurement of a solution of NADH at a known concentration. The phasor representation of the fluorescence decay accounts for the differences in quantum yield of the free and bound form of NADH, pixel by pixel of an image. The concentration of NADH in every pixel in a cell is obtained after adding to each pixel in the phasor plot a given amount of unmodulated light which causes a shift of the phasor towards the origin by an amount that depends on the intensity at the pixel and the fluorescence lifetime at the pixel. The absolute concentration of NADH is obtained by comparison of the shift obtained at each pixel of an image with the shift of the calibrated solution.